

PATENT APPLICATION



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Hiroaki TAKAYAMA, et al.

Appln. No.: 09/214,155

Group Art Unit: 1616

Filed: December 29, 1998

Examiner: Sabiha N. Qazi

For: VITAMIN D3 DERIVATIVE AND ITS PRODUCTION METHOD

LIU DECLARATION UNDER 37 C.F.R. § 1.131

Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Zhaopeng Liu, hereby declare and state:

THAT I am a citizen of P. R. China;

THAT I received a Masters Degree in 1990 from Shandong Medical University, P. R. China and a Ph.D. degree in 2001 from Toyama Medical and Pharmaceutical University, Japan;

THAT I am a member of Japanese Scientific Society related to research in organic chemistry;

THAT I have belonged to Teikyo University, Faculty of Pharmaceutical Sciences, in 1995-1998 as a research fellow, where I have been involved in the synthetic study of Vitamin D₃ in Professor TAKAYAMA's group;

THAT I have been employed by Foreigners Brain Company since 2001, where I have been involved in the synthetic research on new drug candidates.

I have thorough knowledge of the invention in the above-identified patent application, and I have read the non-final Office Action of June 10, 2002 issued in reference to the application. In response to the non-final Office Action, I submit herewith this Declaration, explaining what I observed pertaining to the reduction to practice of the invention claimed in the above-identified application.

Dr. Fujishima was my colleague when I worked in professor TAKAYAMA's group as a research fellow from 1995 to 1998. We understood each other's work very well through daily discussions and

regular group seminars. At the time, one of the main research projects of Professor TAKAYAMA's group was the synthetic and biological study of novel 2-substituted Vitamin D₃. Dr. Fujishima and I were core members of the project. The compounds in the application are a result of that project.

I recall certain incidents which lead me to remember the author and date of experimental notes (1), (2) and (3) very clearly: Dr. Fujishima planned to present the research results of the 2-substituted Vitamin D₃ project at the Tenth Workshop on Vitamin D, so she had to complete the synthesis and measurement of Vitamin D receptor affinity of the compounds in the application prior to the deadline for submission of presentation abstracts, which was prior to March 17, 1997. Dr. Fujishima presented the results of her work on compounds (68) and (72) in a group seminar held prior to March 17, 1997. Exhibit 2, submitted herewith, comprises copies of the handout that Dr. Fujishima distributed at that seminar. I remember seeing and understanding the handout at the time of the seminar.

Page 1 shows a synthesis scheme for compounds #346 and #344, i.e. compounds (68) and (72) respectively, that had been carried out by Dr. Fujishima or under her supervision. The 1st arrow pointing from vitamin D₂ to compound 1 indicates that compound 1 was made from vitamin D₂ by treatment of vitamin D₂ with O₃ (Ozone) and NaBH₄ (Sodium borohydride). The 2nd arrow pointing from compound 1 to compound 2 indicates that compound 2 was made from compound 1 by treatment of compound 1 with TsCl (Tosyl chrolide) in pyridine (Yield 86%). The 3rd arrow pointing from compound 2 to compound 3 indicates that compound 3 was made from compound 2 by treatment of compound 2 with TBSOTf (tert-Butyldimethylsilyl triflate) in 2,6-lutidine (Yield 96%). The 4th arrow pointing from compound 3 to compound 4 indicates that compound 4 was made from compound 3 by treatment of compound 3 with DMSO (Dimethylsulphoxide) and NaHCO₃ (Sodium bicarbonate) (Yield 76%). The 5th arrow pointing from compound 4 to the compound in parenthesis indicates that the compound in parenthesis was made from compound 4 by treatment of compound 4 with n-Bu₄NOH (normal-Tetrabutylammonium hydroxide) in CH₂Cl₂ (Dichloromethane) and H₂O (water). The 6th arrow pointing from the compound in parenthesis to compound 5 indicates that compound 5 was made from the

compound in parenthesis by treatment of the compound in parenthesis with NaBH₄ (Sodium borohydride) and stereoisomer separation by silica gel column chromatography (Yield 45% in 2 steps). The 7th arrow pointing from compound 5 to compound 6 indicates that compound 6 was made from compound 5 by treatment of compound 5 with TsCl (Tosyl chrolide) in pyridine (Yield 93%). The 8th arrow pointing from compound 6 to compound 7 indicates that compound 7 was made from compound 6 by treatment of compound 6 with NaI (Sodium iodide) in DMF (Dimethyl formamide) (Yield 92%). The 9th arrow pointing from compound 7 to compound 8 indicates that compound 8 was made from compound 7 and the compound described above the arrow by treatment of both compounds with n-BuLi (normal-Butyl lithium) and HMPA (Hexamethylphosphoramide) in THF (Tetrahydrofuran) (Yield 72% with recovered 28% of starting compound 7. The yield of this step was increased when using distilled HMPA --- 99%). The 10th arrow pointing from compound 8 to compound 9 indicates that compound 9 was made from compound 8 by treatment of compound 8 with Na-Hg (sodium-mercury amalgam) in THF (Tetrahydrofuran) (Yield 64%). The 11th arrow pointing from compound 9 to compound 10 indicates that compound 10 was made from compound 9 by treatment of compound 9 with TsOH (p-Toluenesulfonic acid) (Yield 85%). The 12th arrow pointing from compound 10 to compound 11 indicates that compound 11 was made from compound 10 by treatment of compound 10 with TPAP (Tetrapropylammonium perruthenate), NMO (N-methylmorpholine) and 4ÅMS (molecular sieves 4 angstrom) (Yield 87%). The 13th arrow pointing from compound 11 to compound 12 indicates that compound 12 was made from compound 11 by treatment of compound 11 with Ph₃P(+)CH₂Br • Br(-) ((Bromomethyl)triphenylphosphonium bromide) and NaHDMS (Sodium hexamethyldisilazide) (Yield 57%). The 14th and 15th arrows pointing from compound 12 to 20epi-Ds (compound #344) and 20epi-Aa (compound #346) indicate that 20epi-Ds and 20epi-Aa were made from compound 12 and a TBS protected compound (described in the upper of the page 5 and 6 of the Exhibit 2 (Ref.1)) by treatment of both compounds with (dba)₃Pd₂CHCl₃, (Tris(dibenzylideneacetone)dipalladium(0)-chloroform adduct),

Ph_3P (Triphenylphosphine) and Et_3N (Triethylamine) in toluene, and then treatment of the resultant compounds with CSA (Camphor sulfonic acid) in MeOH (methanol).

Therefor, I understood that Dr. Fujishima synthesized compounds (68) and (72) prior to March 17, 1997.

Page 2 shows the method for the VDR binding assay. The resultant binding curves for compounds #346, # 344 and 1- α , 25-(OH)₂-VD₃ (control) are also shown. The resultant binding curves of compounds #346, # 344 and 1 α ,25(OH)₂VD₃ (control) are also shown. The upper half of the page shows the method for VDR binding assay which has the same content as the description in the 1st through 3rd pages of Note 3. The bottom half side of the page shows the resultant binding curves which is the same graph described on the 9th page of Note 3.

Thus, I understood that Dr. Fujishima had confirmed the usefulness of compounds (68) and (72) prior to March 17, 1997.

Pages 3 through 6 show the detailed process for synthesizing compounds #346 and #344 from compound #7 as described in scheme 1 on page 1, accompanied by NMR and MS data of each intermediate compound. As for compounds #346 and #344 i.e. compounds (68) and (72), UV data are also shown. Page 3 shows the detailed process for synthesizing compound 8 from compound 7 and compound 9 from compound 8. Page 4 shows the detailed process for synthesizing compound 10 from compound 9, compound 11 from compound 10 and compound 12 from compound 11. Page 5 shows the detailed process for synthesizing 20epiDs (compound #344) from compound 12 and a TBS protected compound. Page 6 shows the detailed process for synthesizing 20epiAa (compound #346) from compound 12 and a TBS protected compound.

From this I understood that Dr. Fujishima had determined a detailed scheme for how to make compounds (68) and (72) and had identified them prior to March 17, 1997.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were

made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: _____

Zhaopeng Liu